IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Atty Docket No. 104385.140)

In Re:
Application of: Bennett et al.

Group Art Unit: 1644

Serial Number: 08/722,659

Examiner: Lubet, M.

Filed: September 27, 1996

For: USE OF HEPARINASE TO DECREASE INFLAMMATORY RESPONSES

#11 Supp 11-24-98

DECLARATION PURSUANT TO 37 C.F.R. §1.131

We, D. Clark Bennett, Elizabeth Cauchon, Ariane Hsia, Pamela Danagher, Brigitte Grouix and Joseph Zimmermann, hereby declare as follows:

1. We are the co-inventors of the above-referenced patent application, which claims priority to provisional application 60/004,622, filed September 29, 1995.

2. We understand that this Declaration is being made to establish a date of invention prior to the date of publication for the following publications which have been cited against the above-referenced patent application by the Examiner:

3. Each of the listed publications was published less than one year prior to the priority date of the instant application.

a. Gilat et al., (1995) J. Exp. Med., vol. 181, pp. 1929-1934, published May 1995.

b. Gilat et al., (1994) J. Immunol. vol. 153, pp 4899-4906, published December 31, 1994

c. Hoogewerf et al., (1995) J. Biol. Chem., vol. 270, pp. 3268-3277, published February 17, 1995.

d. Lider et al., (1995) Proc. Natl. Acad. Sci. USA, vol. 92, pp. 5037-5041, published May 1995.

4. The invention was conceived and reduced to practice in a NAFTA country, Canada, and is entitled to the provisions 35 USC § 104.

5. Prior to the publication dates of the articles recited in paragraph 2 above, we conceived of and reduced to practice the invention which is most broadly claimed in Claim 1 of the above-referenced patent application.

6. Attached hereto as EXHIBIT A, Pages 14 and 15, are true copies of laboratory notebook pages from a bound laboratory notebook, signed by Elizabeth Cauchon and witnessed by Pamela Danagher, except that the dates on the pages have been removed. The redacted dates are all prior to each of the publication dates listed in paragraph 2 above.

7. Notebook Pages 14 and 15 are a record of the reduction to practice of the invention in an *in vitro* neutrophil transmigration assay system. This assay system is an accepted *in vitro* model of neutrophil extravasation and is used to analyze conditions affecting neutrophil extravasation, a key step in local inflammatory response. The results presented in Notebook Pages 14 and 15 demonstrated that heparinase treatment of HUVECs (human umbilical venous endothelial cells) inhibits neutrophil transmigration. The demonstrated inhibition of *in vitro* neutrophil transmigration reasonably infers that a localized inflammatory response in tissue can be decreased by administration of heparinase enzyme to a patient. The results of several neutrophil transmigration assays were presented in the provisional application on pages 26-29 (Example 4).

9. We hereby further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge

that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____
D. Clark Bennett

Dated: _____

Signed: Elizabeth Cauchon
Elizabeth Cauchon

Dated: November 2, 1998

Signed: _____
Ariane Hsia

Dated: _____

Signed: _____
Pamela Danagher

Dated: _____

Signed: _____
Brigitte Grouix

Dated: _____

Signed: _____
Joseph Zimmermann

Dated: _____

that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____
D. Clark Bennett

Dated: _____

Signed: _____
Elizabeth Cauchon

Dated: _____

Signed: Ariane Hsia
Ariane Hsia

Dated: Nov. 8, 1998

Signed: _____
Pamela Danagher

Dated: _____

Signed: _____
Brigitte Grouix

Dated: _____

Signed: _____
Joseph Zimmermann

Dated: _____

that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____
D. Clark Bennett

Dated: _____

Signed: _____
Elizabeth Cauchon

Dated: _____

Signed: _____
Ariane Hsia

Dated: _____

Signed: Pamela Danagher
Pamela Danagher

Dated: November 12, 1998

Signed: _____
Brigitte Grouix

Dated: _____

Signed: _____
Joseph Zimmermann

Dated: _____

that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____
D. Clark Bennett

Dated: _____

Signed: _____
Elizabeth Cauchon

Dated: _____

Signed: _____
Ariane Hsia

Dated: _____

Signed: _____
Pamela Danagher

Dated: _____

Signed: _____
Brigitte Grouix

Dated: 4-11-98

Signed: _____
Joseph Zimmermann

Dated: _____

that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____
D. Clark Bennett

Dated: _____

Signed: _____
Elizabeth Cauchon

Dated: _____

Signed: _____
Ariane Hsia

Dated: _____

Signed: _____
Pamela Danagher

Dated: _____

Signed: _____
Brigitte Grouix

Dated: _____

Signed: _____
Joseph Zimmermann

Dated: Nov 5, 1998

EXHIBIT A

- one plate of HUVEC P7 was trypsinized and 2×10^5 cells were plated per insert (8µm, coated with fibronectin), 29 inserts total. The media was changed every day until they reached complete confluency.

Neutrophil migration assay

Neutrophils migrate in response to a gradient of IL-8. IL-1 stimulated HUVEC cells will produce IL-8 at their cell surface where it presumably binds to heparin. By digesting heparin with our heparinase 3, the IL-8 will diffuse in media and neutrophils, who follow the gradient, will not be attracted to the cell surface, preventing them from migrating into the wells.

One filter on which HUVEC cells were growing was stained with crystal violet (see protocol on page 6). The cells were confluent. Media was taken out of the wells and IL-1 (2ng/ml) was added to 18 wells. The others received culture media only. After 4 hours, the wells were emptied again, along with the inserts and Hep 3 at 1×10^6/ml in PBS was added to both the wells and the inserts, to 9 of them. The others received PBS only. The digestion was allowed to proceed for one hour at 37°C. One insert that received Hep 3 was then stained to see if the cells had detached and it appeared that some had in fact lifted. A different coating is going to be tried next time.

All the inserts were emptied (along with the wells) and 0.3ml of culture media was added to them. The wells and inserts that had some Hep3 received culture media without heparin (RPMI + 20% FS). 1.5×10^6 neutrophils were added to every insert and then migration was

Continued on Page 15

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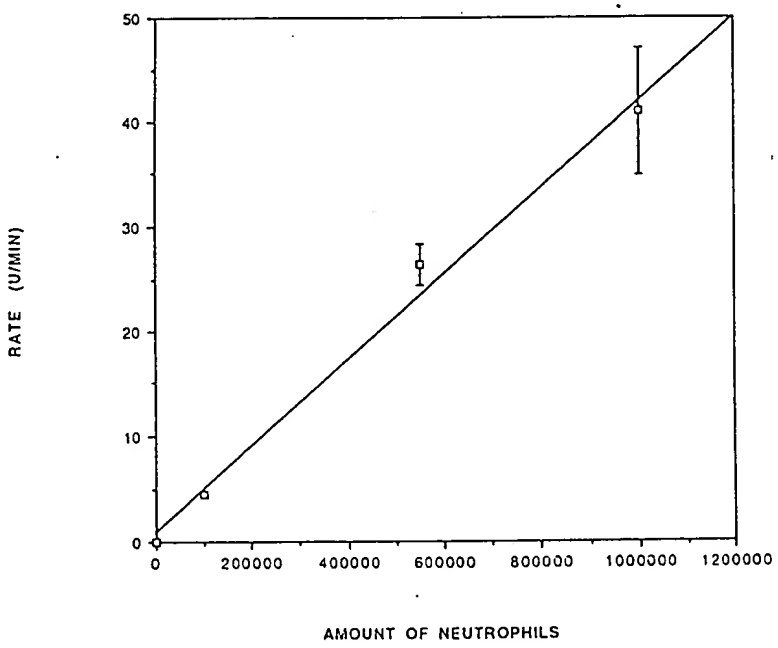
Elizabeth Cauchon — Pamela Hunt

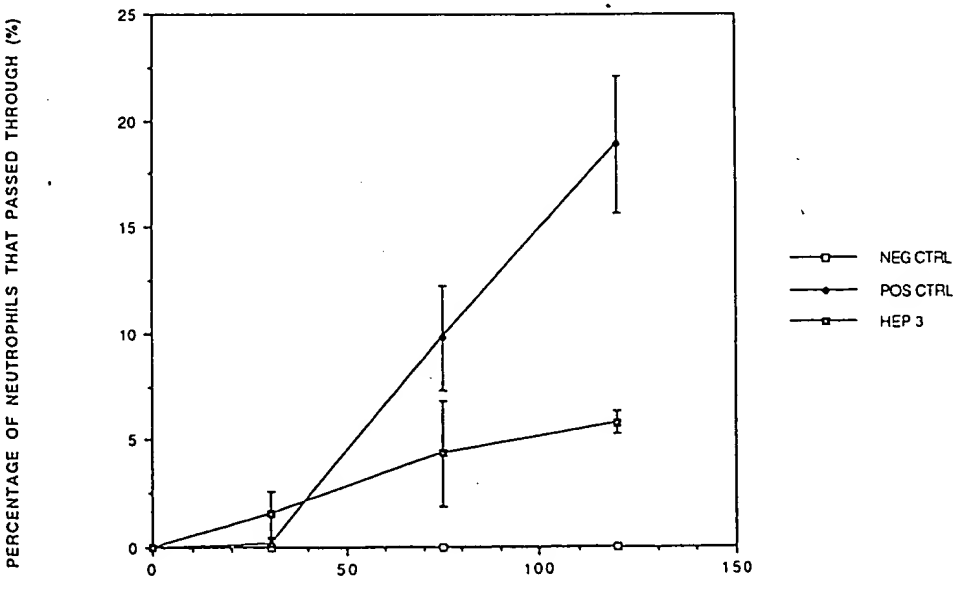
Signed — Date

stopped at 1/2 hr, 1 1/4 hr and 2 hours by taking out the inserts (3 filters of each serie per time point). The bottoms of the inserts were rinsed and the rinse was added to the content of the well. The samples were kept at -20°C O/N and a myeloperoxidase assay was done the day after.

MYELOPEROXIDASE ASSAY OF HUMAN NEUTROPHILS



MIGRATION OF NEUTROPHILS THROUGH A LAYER OF HUVEC CELLS DIGESTED WITH HEP 3 AT 1 IU/ML FOR AN HOUR



Raw Data
Pn Raw
Data Binder
p. 1 to 9

ued on Page 16

Read and Understood By

Elizabeth Cardon _____ Pamela Dangh